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TITLE: Noninvasive Detection of Lactate as a Biomarker of Response Using Spectral-Selective Multiple Quantum Editing Sequence (SS-SelMQC)

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**14. ABSTRACT**

This application focuses on enhancing cancer care by developing non-invasive techniques to determine better biomarkers to improve diagnostic specificity and decrease the number of negative biopsies, and also as markers of response with novel targeted agents such as Trastuzumab and Bevacizumab. Until now, we have constructed radiofrequency coils suitable for studying breast tumors implanted on foot and mammary fat pad, optimized SelMQC, SS-SelMQC, and SEE-SelMQC sequences for lactate/choline detection from whole tumors as well as localized 2D slices with high-resolution using phantoms and part of these methods are tested with *in vivo* MCa-7 tumors.

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None provided.

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## Table of Contents

## Introduction

This application focuses on using lactate as a marker in breast cancer. Until now, choline has been used as a marker with 90% specificity. We propose to use lactate (Lac) as a surrogate marker of breast cancer using our newly developed more sensitive pulse sequence spectral-selective SelMQC 'SS-SelMQC' (1) and SelMQC (2) methods. Lactate detection is technically challenging as breast tissue has very high levels of lipid (Lip) and water. Treatment of breast cancers with novel targeted agents such as Trastuzumab and Bevacizumab have led to significant gains, although the drugs can be toxic. Breast tumors are usually sensitive to many drugs but subsequently develop resistance. There is strong interest in applying drugs that interfere with angiogenesis and signaling pathways related to breast cancer growth and metastasis. Low extracellular pH and high Lac levels were shown to be indicators of metastatic risk in breast cancer xenografts. Elevated lactate in biopsy samples was shown to correlate with increased risk of metastasis and poor patient survival in different aggressive cancers, while a decrease lactate levels observed in tumor response to radiation and chemotherapy. Therefore, non-invasive measurement of Lac may be an additional characteristic marker for breast cancer; it may improve diagnostic specificity, serve as an early marker of tumor response, and provide functional information about prognosis.

## Body

The initial phase of the proposal focuses on developing and evaluating a more effective method of detecting lactate non-invasively by magnetic resonance (MR) techniques. Lac is present in very small quantities compared to fat and water and the signal of Lac occurs in the same position as Lip, thereby making it very difficult to see. We have implemented recently developed a sequence (1) which on preliminary testing has been shown to be far more sensitive than prior methods (2). The preliminary data showing the efficacy of this sequence was demonstrated using R3327 prostate tumors. We plan to more fully evaluate this sequence and ensure that it will detect lactate in mice which is more difficult due to smaller volumes within the localized slice. We will also implement SEE-SelMQC (3) technique to simultaneously detect lactate and choline (Cho), a compound that is currently used as a tumor marker in breast, brain and prostate imaging studies. This will serve as a starting point for SS-SelMQC modification for simultaneous detection of lactate and choline resonances with improved sensitivity.

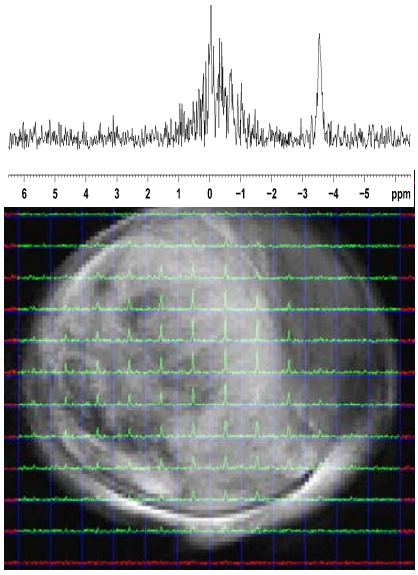


Figure.1. lactate spectra from whole tumor (top) as well as 2D localized CSI slice (botom). The breast coil diameter is 16 mm, which is ideal for breast tumors at higher volumes proposed in this study. We optimized the SelMQC sequence for in-plane resolution of 1.25 x1.25 $mm^2$ using FOV = 20mm at 7T. Slice thickness of 5 mm used in this study. TR = 2s; Number of scans=4; tumor volume is 727 $mm^3$ and acquisition time = 32 mins;

Research is focused on:

1) Optimizing RF coils suitable for breast tumors implanted in leg or mammary fat pad at low and higher tumor volumes proposed in this study.

2) Implementing and evaluating the newly proposed lactate detection sequence using these breast RF coils specifically tailored for mice tumors and compare with

other sequences. Sequences are compared in terms of signal to noise ratio.

3) Evaluating localization spatial profiles using phantoms of different concentrations with optimized voxel sizes and testing with *in vivo* tumors.
4) Evaluation of tumor volume measurements using MRI.
5) Implementation and evaluation of sequence for simultaneous detection of choline and lactate resonances from whole tumors.

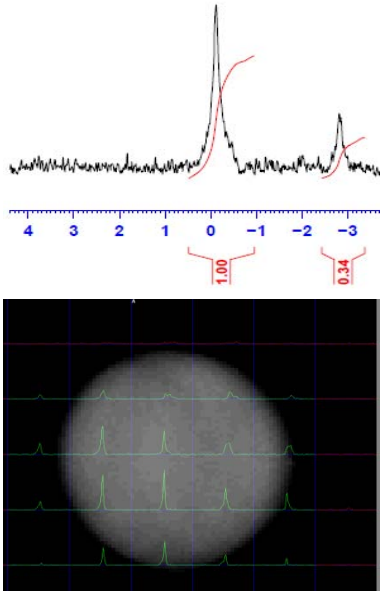


Figure.3. shows Lac from whole tumor (top) as well as 2D localized CSI slice (bottom) obtained using SS-SelMQC. The breast coil diameter is 11 mm ideal for breast tumors <u>at lower tumor volumes</u> proposed in this study. We optimized the SS-SelMQC and SelMQC sequence for in-plane resolution of 2.5x2.5mm$^2$ using FOV=20mm with 8 x 8 matric size. Data collected at 4.7T. Slice thickness of 5mm used in this study. TR=2s; Number of scans=4; acquisition time=32 mins; As seen from Fig. 3A the integral of SS-SelMQC peak (left peak of top)obtained from whole tumor is 3 times higher than SelMQC (right peak of top).

The issues of implementing the pulse sequences for optimizing to create useful metabolite images and construction of radiofrequency coils are closely connected. In the application, we presented preliminary data showing in vivo detection of lactate in MCa7 tumor models in mice. We have designed different coil sizes optimum for different breast tumor sizes proposed in this study. We are also working on $B_1$ inhomogeneity profiles of RF coils designed. We did a lot of our pulse sequence optimization with 2-turn solenoid coils.

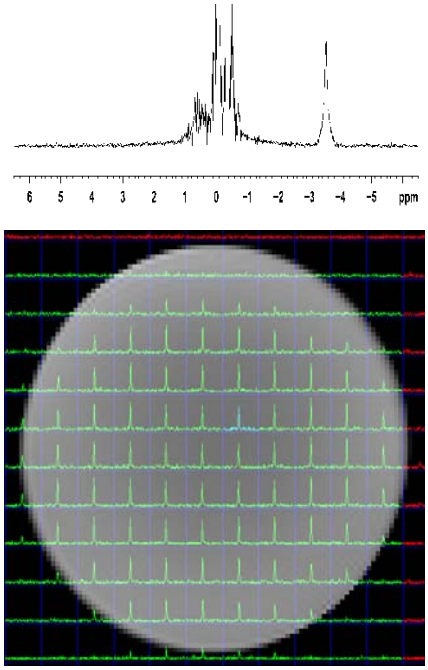


Figure.2. Lac spectra from whole tumor (top) as well as 2D localized CSI slice (bottom) using 10mM Lac in $H_2O$ with 10% saline. This data is produced with the same breast coil and parameters used for testing *in vivo* tumor shown in Figure 1.

<u>Lac detection using SelMQC and SS-SelMQC methods:</u> We have been able to selectively detect lactate using SelMQC (Selective Multiple Quantum Coherence) (2) in non-localized mode (Figure 1A) and slice localized 2D chemical shift image data optimized for studying tumors at higher tumor volumes although some further optimization is necessary with other sequence (1) with respect to spacial profile optimization. Even with localization, the data quality is very good and signal looks more uniform. Before obtaining this invivo data, we tested the lipid suppression with Lac-Lip phantom and further lactate detection was optimized for desired resolution which is possible to achieve using 10mM Lac phantom with water and 10% saline solution without Lip. This is very important issue towards understanding tumor heterogeneity. Figure 2 shows both non-localized (A) and localized spectra of Lac (B). SS-SelMQC and SelMQC methods were compared using smaller diameter (11 mm) breast coil and were able to reproduce approximately

2-3 times signal enhancement of Lac in SS-SelMQC in comparison to SelMQC (Figure 3 (top)). Soon we will be verifying this result with invivo tumors.

Tumor Measurements: In the conventional method, tumor volume is measured using equation $\pi$ /6 * l* b * h, where l, b, and h are length, breadth and depth respectively. This method yielded a value (737mm$^3$) and was compared with MRI tumor volumes. Although both the measurements found to be consistent for MCa foot tumors due to its location, we propose to measure tumor volumes accurately using MRI for breast tumors implanted on mammary fat pad. MRI may be a method of choice than conventional way due to tumor location which will be further evaluated. MRI tumor volume measurements were carried out with $T_2$ – weighted spin echo 2D MRI images. The tumor size was assessed in each serial MR images (Figure 4) by region of interest (ROI) based measurements. For ROI based measurement, the tumor area, i.e., the ROI, was traced on the computer work station with a polygon in each axial $T_2$-weighted images (ROIs are shown in green). ROI-based volumes were calculated by the summation of all tumor areas in each slice and multiplication by the slice profile.

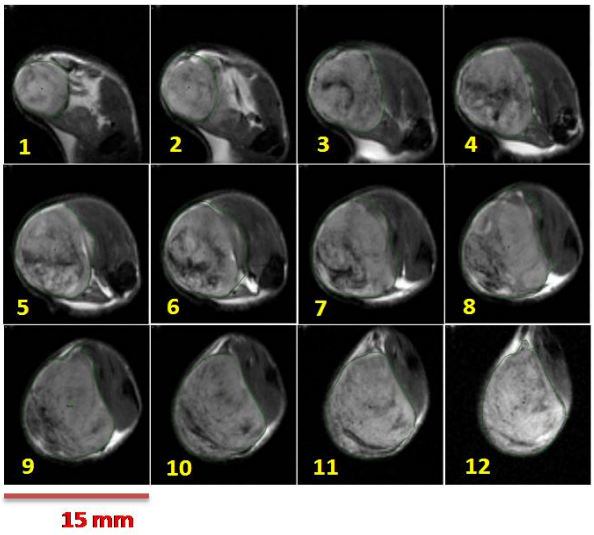


Figure.4. These images were obtained using spin echo (SE) pulse sequence with TR/TE = 2000/50 ms, slice thickness = 1mm, inter slice thickness 1mm, field of view (FOV) = 24×24mm$^2$, matrix = 128 × 128, 4 averages and total measuring time 4 min.MRI tumor volume is 727mm$^3$.

Simultaneous detection of Lac and Cho: We have implemented the SEE-SelMQC (3) for simultaneous detection of Lac and Cho resonances in a single-shot. This preliminary data was generated using in vivo MCa tumors and compared with SelMQC sequence (Figure 5). This was also tested using 10mM Lac and Cho in 90% water and 10% saline. As we see from Figure 5, choline and lactate resonances were detected with excellent water suppression. We have some technical issued with slice localization in this sequence as this pulse is 180$^o$ pulse. Recently, we optimized the localization with different pulse shapes and finalized it with a broadband refocussing 'Mao' RF pulse (4) as a refocussing slice selective pulse and further optimization and processing underway to check the localization. We are very optimistic about evaluating this sequence even in the 2D localized mode soon.

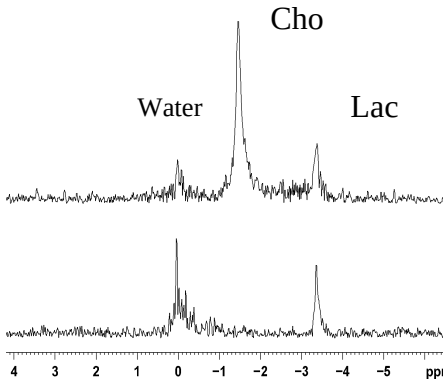


Figure 5. MR Spectra of Cho and Lac resonances from Mca7 foot tumor. Simultaneous detection of Lac and Cho was detected (top). Similar Lac signal observed from SelMQC sequence (bottom). The breast coil diameter is 16 mm, which is ideal for breast tumors at higher volumes proposed in this study. We optimized the SEE-SelMQC sequence TR=2s; Number of scans=16; tumor

Initial phase of technical goals have been achieved or are fairly close with slow progress with *in vivo* tumor preparation. There is a new postdoctoral fellow who arrived 2 months back and obtained his animal handling clearance and started working on this project full time and

we are optimistic that this will help achieve progress in a timely style.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

1. We can now obtain high resolution 2D chemical shift image of Lac from a localized slice with high resolution using SelMQC editing sequence.

2. Reproducibility of Lac signal enhancement and suppression of water and Lip levels using SS-SelMQC, in comparison with SelMQC sequence.

3. Technology for simultaneous detection of Cho and Lac was implemented using SEE-SelMQC and tested with phantoms and *in vivo* tumors from whole sample.

REPORTABLE OUTCOMES:

None

CONCLUSION:

We made progress with technical implementation and evaluation of sequences. We are slightly behind schedule with *in vivo* work but clearly making progress. We are hopeful that within next month, breast tumors will be implanted and studied.

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## Appendices

None

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Provide the following information for the key personnel in the order listed on Form page 2.
Photocopy this page or follow this formate for each person

| NAME | POSITION TITLE |
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EDUCATION TRAINING *(Begin with Baccalaureate or other initial professional education, such as nursing and include postdoctoral training)*

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|---|---|---|---|
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| Gulbarga University, Gulbarga | MS | 1996 | Chemistry |
| Gulbarga University, Gulbarga | Ph.D | 2007 | Chemistry |

**A. Positions and Honors**

**Professional Experience**

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2002-2005 Reserarch Studentship of Gulbarga University, Gulbarga India
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2007-2009 Visiting Associate, Centre of Biomedical Magnetic Research, Lucknow, India
2008 Educational Stipend award for attending ISMRM conference

**B. Selected Publication**

Annarao, S., Sidhu, O.P., Roy, R., Tuli, R. and Khetrapal, C.L. (2008). Lipid profiling of developing Jatropha curcas L. seeds using $^{1}$H-NMR Spectroscopy. *Bioresour Technol*. 99: 9032-9035.

Mahdi, A.A., Annarao, S., Tripathi, S., Nawab, A., Mahdi, F., Mahdi, H., Roy, R. and Khetrapal, C.L. (2008). Age related Metabonomic changes in the serum and uninary composition of male Sprague-Dawley rats effect on cognitive function. *Open Magnetic Resonance*. 1: 71-76.

Sidhu, O.P., Annarao S., Pathre Uday., Snehi, S.K., Raj, S.K., Roy Raja., Tuli Rakesh. And Khetrapal, C.L. (2010). Metabolic and Histopathological alterations of Jatropha Mosoic begamovirus infected Jatropha Curcus L. by HR-MAS NMR Spectroscopy and Magnetic Resonace Imaging. *Planta.* 232: 85-93